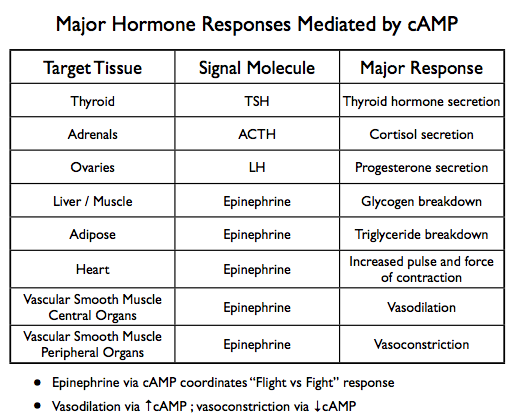
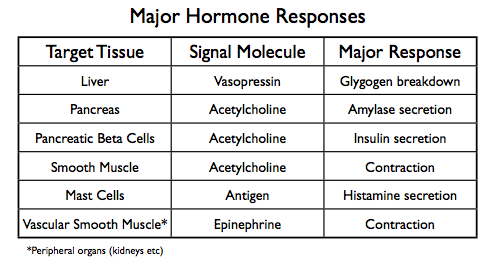
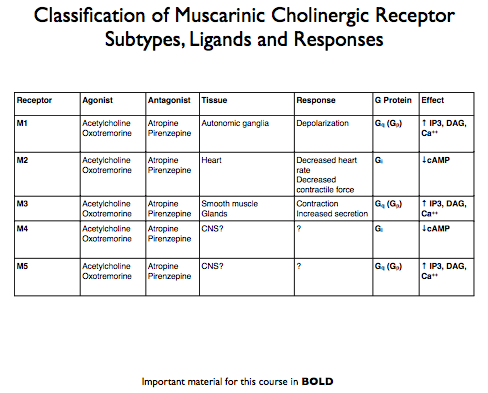
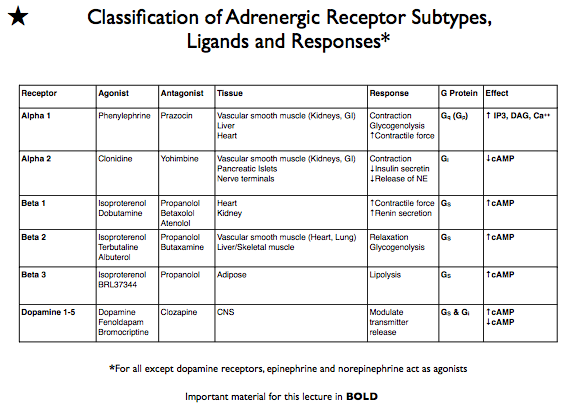
SIGNAL TRANSDUCTION & DRUG DISCOVERY

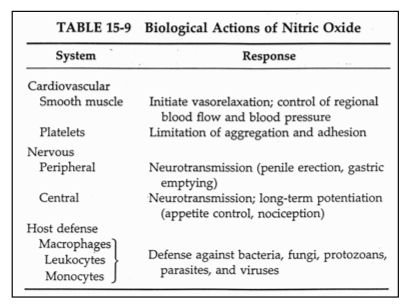
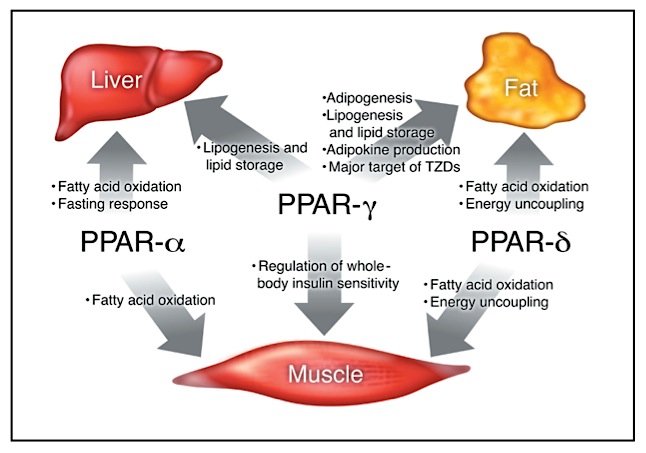
1. **Know the mechanism by which Gleevec blocks growth of chronic myelogenous leukemia through the receptor tyrosine kinase pathway** 
   1. In Chronic Myelogenous Leukemia patients they have the chromosome translocation 9:22 (Philadelphia translocation)—the Tyrosine Kinase (ABL) on chromosome 9 moves to chromosome 22 where it form a fusion protein, Bcr-Abl which is constitutively active. This unregulated activity leads to constitutive activation of the Ras-MAP kinase pathway which leads to unregulated growth and thus malignancy.
   2. Gleevec is a drug that targets this Bcr-Abl fusion protein and inactivates it which turns off the growth pathway. 95% survival rate of patients at 60 months with CML who took Gleevec.
   3. Similarly in Melanoma patients 50% have a B-raf (serine/threonine kinase) mutation leaving it constitutively active. The drug Zelboraf that targeted this mutated kinase and inactivated it showed an 80% tumor regression vs. 5% for chemotherapy.
2. **Know the major steps of G-protein signaling for the cAMP and phosphoinositide pathways** 
   1. Ligands for G protein linked receptors
      1. Catecholamines
      2. Neurotransmitters
      3. Peptide hormones
   2. Second messengers produced
      1. cAMP
      2. DAG
      3. IP3
      4. Ca++
      5. cGMP
   3. Three Major classes of G protein-linked receptors
      1. cAMP pathway (Gs ↑ cAMP & Gi ­ ↓ cAMP)
      2. Phospholipase C Pathway (Gp/Gq)—affects DAG, IP3, and Ca++
      3. Rhodopsin Pathway (GT)— ↓ cGMP—works in the eye
   4. cAMP Pathway
      1. Agonist comes and binds a G protein linked receptor
         1. Depending on what kind of G-protein linked receptor is activated determines this response
         2. β -adrenergic receptor vs. α2 adrenergic receptor
            1. when Epinephrine binds both of these they do opposite things but work together in FIGHT and FLIGHT response (concentrating blood flow where it is needed)
      2. G protein gets activated and activated adenylyl cyclase (AC)
         1. Gs—increases cAMP
            1. Epinephrine binding to β receptors of heart vasculature activate AC and increase cAMP which causes vasodilation to increase blood flow
         2. Gi—decreases cAMP
            1. Has β and γ subunits identical to those in Gs­
            2. Has a distinct αi that binds and hydrolyzes GTP but inactivates AC activity
            3. Epinephrine binding to α2 adrenergic receptors in peripheral organ vasculature (kidney, GI)—vasoconstrict to decrease blood flow

PKA phosphorylates and inactivates Myosin Light Chain Kinase

* + 1. AC converts ATP 🡪 cAMP
    2. cAMP activates cAMP-dependent protein kinase (R2C2= PKA)
    3. PKA is a tetramer with 2 cAMP binding chains and 2 catalytic chains
    4. When 2 cAMP bind PKA it releases the 2 catalytic subunits which go and phosphorylate substrates (usually enzymes) to produce a response
       1. With Epinephrine induced breakdown of live and muscle glycogen the activated PKA phosphorylates phosphorylase kinase to activate it and that then goes to activate glycogen phosphorylase which converts glycogen to glucose-1-phosphate which can enter glycolysis
       2. During this process activated PKA also phosphorylates the enzyme (glycogen synthase) needed for glycogen synthesis but in this case it **inactivates** it.
    5. To regulate—dephosphorylation of substrates by phosphatases OR degradation of cAMP by phosphodiesterase (PDE) into 5’-AMP
  1. ****Ca++/Phosphoinositide Signal Pathway
     1. Produces Ca++, IP3, DAG
     2. This pathway is controlled by Gq (G protein)
        1. Structure is similar to Gs-- αq interacts with receptor and activates effector enzymes while β/ γ dimer are used to anchor the complex to the membrane
     3. GTP-Gq activates phospholipase C (PLC)
     4. PLC cleaves PIP2 to yield DAG (lipophilic) and IP3 (hydrophilic)
     5. DAG stays in PM to activate PKC leading to an intracellular phosphorylation cascade
        1. **Phorbol esters** mimic DAG
     6. IP3 moves to cytoplasm to trigger Ca++ release from intracellular stores leading to Ca++/calmodulin cascade
        1. **Calcium ionophores (Ionomycin**) mimic IP3
     7. Ca++ binds calmodulin (CaM) which regulates multiples enzymes and proteins:
        1. MLCK (phosphorylates myosin causing interaction with actin and muscle contraction—ACh induction of SM contraction)
        2. NO synthase (NOS)—produces NO causing relation of vascular SM and vasodilation (ACh action at vascular endothelium)

1. **Be able to identify the different types of adrenergic and cholinergic G- protein signaling pathways, with an emphasis on Receptor Type, G- protein subclass, and 2nd Messengers produced.** 
   1. Protein G-receptors of the CNS
      1. Adrenergic = epinephrine/norepinephrine
      2. Cholinergic= acetylcholine



1. **Know mechanism of nitric oxide signaling and the action for Sildenafil Citrate (Viagra).** 
   1. ****
   2. NO signaling
      1. NOS= Nitric Oxide Synthase🡪a Ca++/CaM-dependent enzyme
      2. NO diffuses from endothelium cells of BV’s to adjacent SM
      3. NO activates soluble Guanylate Cyclase (sGC) elevating cGMP
      4. cGMP causes dephosphorylation of myosin light chains, causing relaxation and vasodilation
      5. Nitrovasodilators (nitroglycerin) directly activates sGC
      6. PDE blocks response by converting cGMP 🡪 inactive 5’GMP
   3. Action for Sildenafil Citrate (Viagra)
      1. PDE5 is the major PDE of the corpora cavernosa of the male genitals
         1. Sildenafil citrate (Viagra) is a potent selective inhibitor of PDE5 which degrades cGMP in the corpus cavernosum – Viagra prevents this degradation of cGMP thus preventing relaxation of SM (vasodilation) of helicine arteries which causes an erection to last longer
      2. PDE3 is the major PDE of the human heart
      3. PDE6 is major isoenzyme of the retina (only a 10 fold selectivity for PDE5 over PDE6)
2. **Know major steps of nuclear receptor signaling** 
   1. Nuclear receptor ligands—derivatives of cholesterol, aromatic amino acids or FA
      1. Human genome contains 48 Nuclear Receptor Genes
   2. Major Steps (2)
      1. Ligand binds receptor which recruits the steroid receptor co-activator complex (CoA) containing enzymes with histone acetyl transferase (HAT) activity—acetylates (A) histones
         1. Steroid/Thyroid Receptor Superfamily
            1. Has transcription activation domain (promotes activity by DNA polymerase), a DNA binding domain, Ligand binding domain (site of high selectivity binding by steroid hormones)
      2. Acetylation leads to unwinding of chromatin allowing binding by general TF’s (Pol II)
3. **Understand the concepts of Orphan Nuclear Receptors and Reverse Endocrinology** 
   1. Orphan (Nuclear) Receptors
      1. an apparent receptor that has a similar structure to other identified receptors but whose endogenous ligand has not yet been identified
      2. Ex: EER1, EER2, HAP
      3. ~30 have been discovered
      4. Orphan receptors lack known ligands but that represent candidate receptors for new hormones. Recently, natural and synthetic ligands have been identified for several orphan receptors and used to dissect their biological roles. This “**reverse endocrinology**” strategy has resulted in the discovery of unanticipated nuclear signaling pathways for retinoids, fatty acids, eicosanoids, and steroids with important physiological and pharmacological ramifications.
   2. Recently discovered Orphan Receptor Ligands
      1. Liver X Receptor (LXR (α, β))—the cholesterol sensor
         1. Expressed in metabolic tissues: liver, adipose, kidney, intestines and macrophages
         2. Activated by cholesterol metabolites (oxysterols)
         3. Control cholesterol homeostasis
            1. Transport, catabolism and excretion of cholesterol
      2. FXR (Foresaid X Receptor—the bile acid sensor)
         1. Expressed in live and intestine
         2. Activated by bile acids (cholic acid and derivatives)
         3. Controls bile acid efflux from liver and excretion at intestines
            1. Serve to solubilize lipids and vitamins for excretion
      3. How they work together to promote cholesterol excretion
         1. LXR controls
            1. Cholesterol efflux from peripheral tissues (e.g., macrophages)
            2. Metabolism at liver to bile acid or esterified cholesterol
            3. Efflux of cholesterol to intestines for excretion
         2. FXR
            1. Regulates bile acid metabolism at liver
            2. Efflux from liver to serve as detergent for lipid (cholesterol) excretion
4. **Know mechanism by which Rosiglitazone and related TZD drugs control PPARγ in the treatment of diabetes** 
   1. Type 2 Diabetes
      1. High glucose 🡪 insulin promotes glu uptake and storage as glycogen in liver and muscle as well as inhibits lipolysis at adipose (reducing serum FFAs)
      2. In excess FFA, lipid buildup in muscle and liver desensitizes the organs to insulin action, causing insulin resistance and hyperglycemia
      3. Thus redistribution of FFAs to adipose is beneficial in treatment of diabetes
      4. ^^ is achieved through FFA and drug activation of PPAR γ
   2. PPAR= Peroxisome Proliferator-Activated Receptor
   3. 
      1. PPARα
         1. Expressed in liver and muscle
         2. Activated by fatty acids and fibrate drugs
         3. Hypolipidemic actions
            1. Promote FFA uptake, Beta-oxidation of FAs
      2. PPARγ
         1. Expressed in liver, muscle, adipose and bone
         2. Activated by
            1. fatty acids and arachidonic acid derivatives
            2. anti-diabetic drugs **– thiazolidinediones (TZDs**)
         3. Insulin sensitizing actions
            1. Promote **adipocyte differentiation, lipogenesis and storage**
            2. Promote **glucose uptake** in liver and muscle
            3. Multi-billion dollar drugs!
      3. PPARδ
         1. Ubiquitous expression but low abundance
         2. Little known, but may have effects similar to PPARγ
   4. Rosiglitazone (Avandia) is a TZD (which activate PPAR γ) and used for treatment of type II diabetes

